Recombination frequency

1. **Introduction of recombination frequency**
2. **Calculation of recombination frequency**
3. **Relation between recombination frequency and map unit**
4. **Introduction to Genetic mapping**
5. **Linkage groups**
6. **Gene mapping from two point test cross**
7. **Gene mapping from three point test cross**
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Recombination frequency

- It is simply called **Percentage of Recombination**
- **Recombination frequency**= $\frac{\text{Number of recombinants}}{\text{total number of progeny}} \times 100$
- **1% Recombination** = 1 cross over occurred in every 50 cells undergoing meiosis
- If 1 meiotic cell in 50 has a crossover, the percentage of crossing over= 1/50= 2%
- **2% crossing over** = among 200 chromosomes that result from meiosis in 50 cells exactly 2 chromosomes (those involved in exchange) are recombinant for genetic marker flanking that cross over

2% CROSS OVER= 1% RECOMBINATION

Calculation of recombination frequency

Consider a cross between 2 different traits affecting pod characteristics in garden pea plant i.e. **Narrow, yellow pods** (**aabb**) and **Normal, green pods** (**AaBb**)

The F1 progenies (**AaBb**) were then testcrossed to plants with narrow and yellow pods (**aabb**). The progenies resulting from this cross listed in the table:

Class	Phenotype	Number
1	Normal and green pods (AaBb)	445 parental types
2	Narrow and yellow pods (aabb)	455 parental types
3	Normal and yellow pods (Aabb)	51 recombinant types
4	Narrow and green pods (aaBb)	49 recombinant type

Normal green pod **AaBb** × Narrow yellow pod **aabb**

F1: **AaBb** × **aabb**

Normal and green pods (AaBb)

Narrow and yellow pods (aabb)

Normal and yellow pods (Aabb)

Narrow and green pods (aaBb)

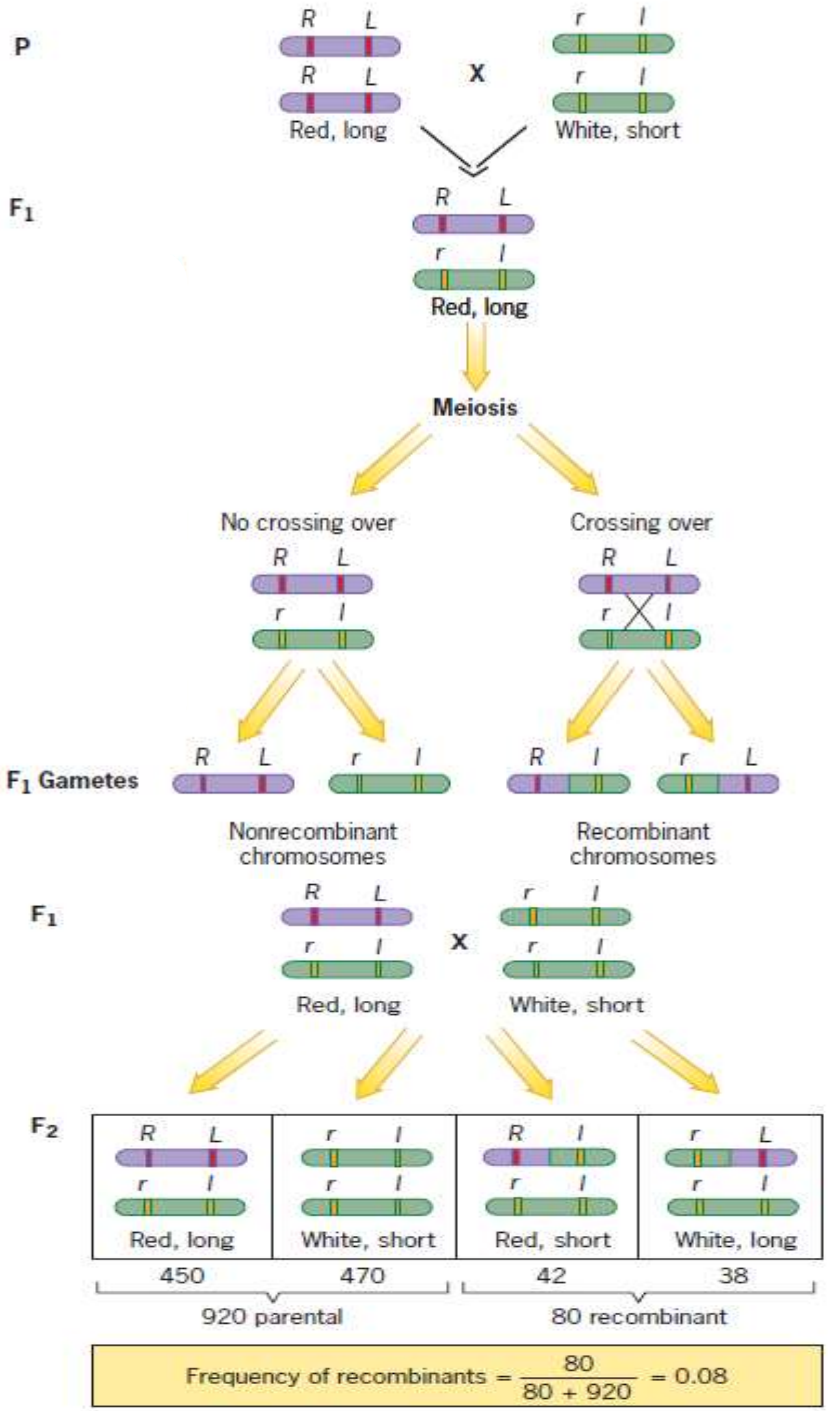
Recombination frequency = $\frac{\text{Number of recombinants}}{\text{total number of progeny}} \times 100$

- So to calculate recombination frequency, we need number of recombinants, total number ob progeny according to the formula
- According to given data in the table,
- Number of recombinants= 51+49
- Total number of progeny= 445+455+51+49
- Substitute to the formula,

$$\frac{51+49}{445+455+51+49} \times 100 = \frac{100}{1000} \times 100 = 10\%$$

Class	Phenotype	Number
1	Normal and green pods (AaBb)	445 parental types
2	Narrow and yellow pods (aabb)	455 parental types
3	Normal and yellow pods (Aabb)	51 recombinant types
4	Narrow and green pods (aaBb)	49 recombinant type

Therefore, the recombination frequency in this case= 10%



Consider 2 traits of flower namely color of flower and pollen length.

Cross was made between Red and long pollen flower of genotype (RRLL) with White and short flower of genotype (rrll)

F1 generation progeny were Red and long flower with genotype (RrLl)

On crossing over produced recombinant chromosomes

In F2 generations it produced:

Class	Phenotype	Number
1	Red long flower (RrLl)	450 Parental types
2	White short flower (rrll)	470 Parental types
3	Red short flower (Rrll)	42 Recombinant types
4	White long flower (rrLl)	38 recombinant types

Number of recombinants= 42+38

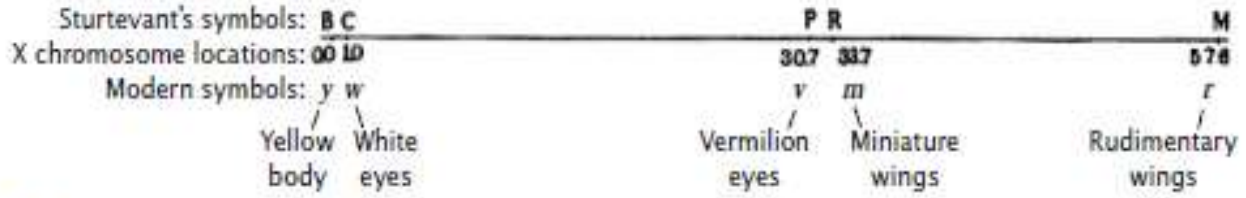
Total number of progeny= 450+470+42+38

Recombination frequency= 0.08✕100= 80%

Genetic mapping

- Linkage of genes in a chromosome can be represented in the form of genetic map (linkage map/ chromosome map)
- Alfred Sturtevant- first to construct the genetic map

- He used the frequency of crossing over (i.e. recombination frequency) between two genes to prepare the first genetic map of X-chromosome of Drosophila
- 2 major aspects to genetic mapping:
  - the determination of the linear order with which the genes are arranged with respect to one another (gene order)
  - The determination of the relative distance between the genes (gene distance)


Sturtevant's symbols: B C P R M
X chromosome locations: 0.0 1.0 30.7 33.7 57.0
Modern symbols: y w v m r
Yellow body
White eyes
Vermilion eyes
Miniature wings
Rudimentary wings

7.1 Sturtevant's map included five genes on the X chromosome of *Drosophila*. The genes are yellow body (*y*), white eyes (*w*), vermilion eyes (*v*), miniature wings (*m*), and rudimentary wings (*r*). Sturtevant's original symbols for the genes are shown above the line; modern symbols are shown below with their current locations on the X chromosome.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 160

- The percentage of recombination (Recombination frequency) can be used to represent a measure of distance (map distance) between 2 genes
- The percentage of recombination (crossing over) between 2 loci is directly related to the physical distance between the loci
- 1% Crossing over/ Recombination = 1 map unit / 1 cM

$$\text{Map distance} = \frac{\text{Number of recombinant offspring}}{\text{total number of offspring}} \times 100$$

- The unit of measurement for genetic map is known as map unit or centimorgan (Cm)
- 1 map unit/ 1cM= 1% Recombination
- Thus for example 2 genes having recombination frequency of 3.5% is said to be located 3.5 map units apart

For example,

Suppose 2 genes (**A** and **B**) are located on same chromosome. An **AABB** individual is crossed to an **aabb** individual to produce **AaBb** offspring. The **AaBb** offsprings are then testcrossed to **aabb** individuals.

Let us assume this cross produces total 400 offsprings. Among them 325 offsprings are parental and 75 are recombinant

P1 **AABB** × **aabb**

Gametes **AB** **ab**

F1 progeny **AaBb**

Test cross **AABB** × **aabb**

Gametes **AB** **ab** **Ab** **aB** **ab**

F2 progeny

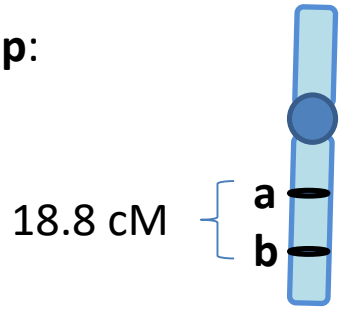
ABab	160 offsprings	Parental
abab	165 offsprings	
Abab	36 offsprings	Recombinant
aBab	39 offsprings	

Recombination frequency =

$$\frac{36+39}{36+39+160+165} \times 100 = 18.8\text{cM}$$

The genes are approximately 18.8 cM apart

Genetic map:



Linkage group

All genes present on a particular chromosomes form a linkage group

Number of linkage group of a species corresponds to the total number of different chromosomes of that species

In humans,

male= 22 pairs autosomes+ 1X chromosome+ 1Y chromosome= 24 linkage groups

female= 22 pairs autosomes+ 2X chromosomes= 23 linkage groups

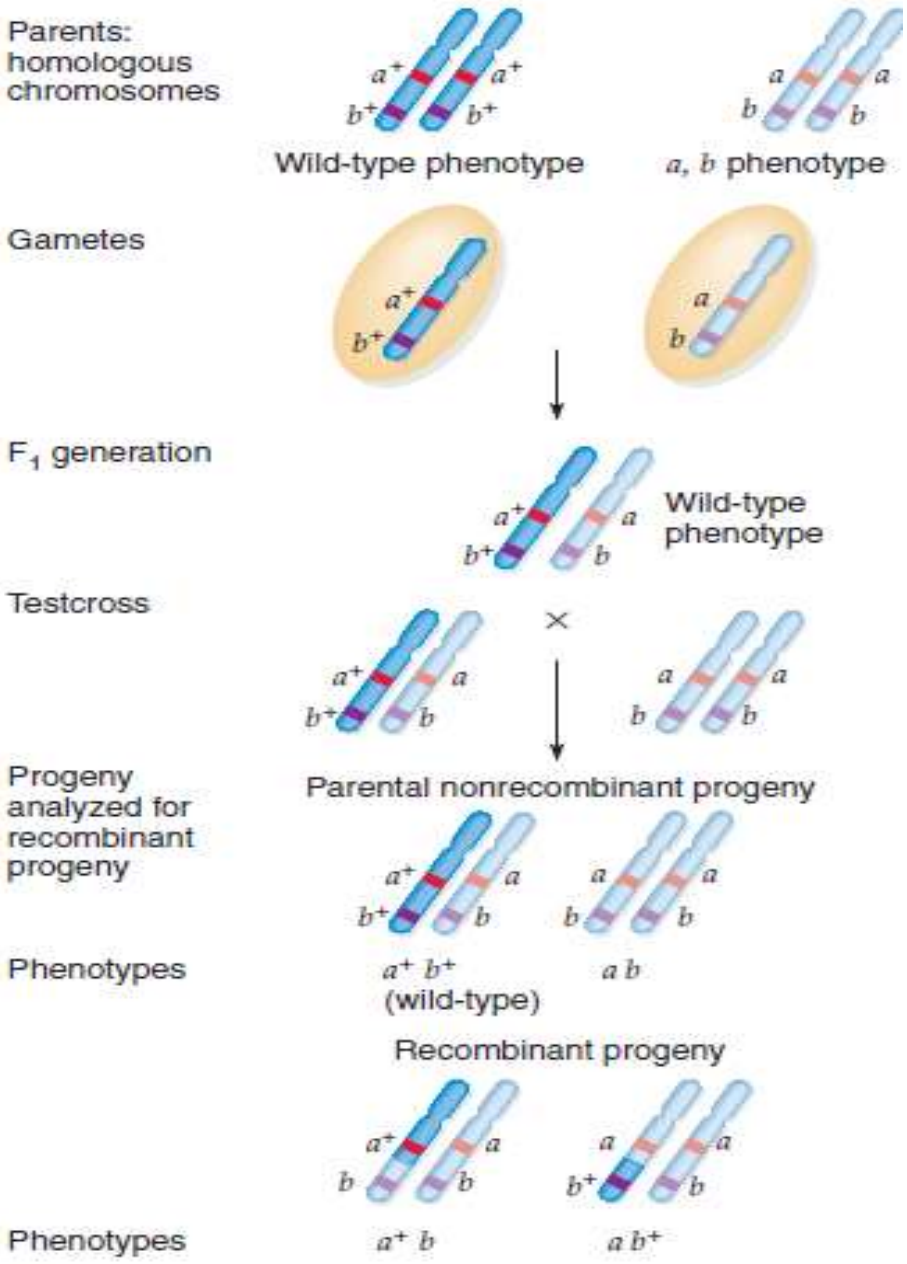
Gene mapping from two point cross

A cross involving two loci is usually referred to as a two point cross

Gene mapping from two point cross can be understood with the help of following examples,

Example 1: A double heterozygote is crossed with doubly homozygous recessive mutant strain. When double heterozygous **a^+b^+/ab** F1 progeny from a cross of **a^+b^+/a^+b^+** with ab/ab are testcrossed with ab/ab, four phenotypic classes are found among the F2 progeny.

Two of these classes have the parental phenotypes **a^+b^+** and **ab**, and the other two have the recombinant phenotype **a^+b** and **ab^+**



Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.407

Class	Phenotype	Number
1	**a^+b^+**	445 Parental type
2	**ab**	455 parental type
3	**a^+b**	51 recombinant type
4	**ab^+**	49 Recombinant type

1st step: find out the Recombination frequency

RF= $\frac{100}{100} \times 100 = 10\%$

2nd step: Interpret the result

The recombination frequency between two loci is directly related to the physical distance between the loci

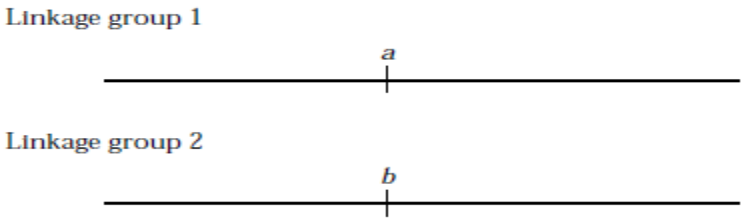
1% crossing over/ Recombination= 1 map unit/ 1cM

So **Map distance**= 10cM

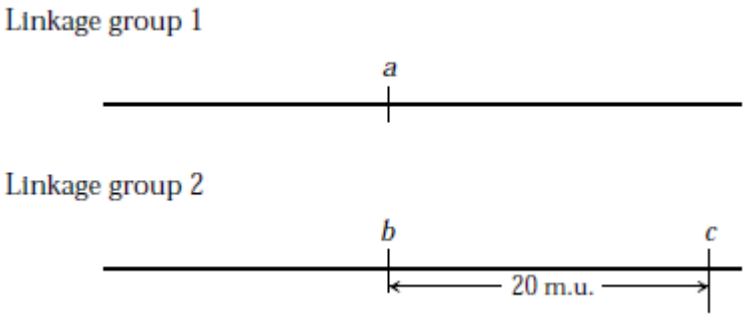
Example 2: Suppose we carried out a series of two-point testcrosses for four genes, a,b,c and d and obtain the following recombination frequencies:

Gene loci in testcross	Recombination frequency (%)
a and b	50
a and c	50
a and d	50
b and c	20
b and d	10
c and d	28

1. Consider recombination frequencies for each pair of genes
2. Recombination frequency of (**a** and **b**) and (**a** and **c**)= 50%
   - therefore it is RF expected with independent assortment
   - therefore gene (**a** and **b**) and (**a** and **c**) are on different chromosomes so placed in different linkage group

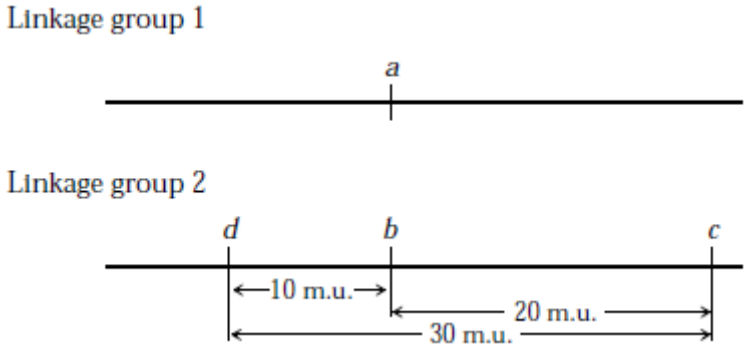


3. Recombination frequency of (**b** and **c**)= 20%
   - Therefore (**b** and **c**) are linked and present on chromosome with 20 mu apart



4. The recombination frequency between (**a** and **d**) = 50%
    - therefore these genes are not linked and present in different linkage group
5. Recombination frequency between (**b** and **d**) = 10%
    - Therefore these genes are linked and present 10 mu apart
6. To decide whether gene **d** is 10 mu to left or right of gene **b,** we must consult the **c** to **d** distance
7. If gene **d** is 10 mu to left of gene **b**, then the distance between **d** and **c** should be 20 mu+ 10 mu= 30 mu
8. If gene **d** lies to right of gene **b**, then distance between gene **d** and **c** will be much shorter approximately, 20mu-10mu= 10 mu
9. By examining RF of **c** and **d** we can conclude the correct gene order
10. RF (**c** and **d**)= 28% = 28mu
11. So gene **d** must lie to the left of gene **b**

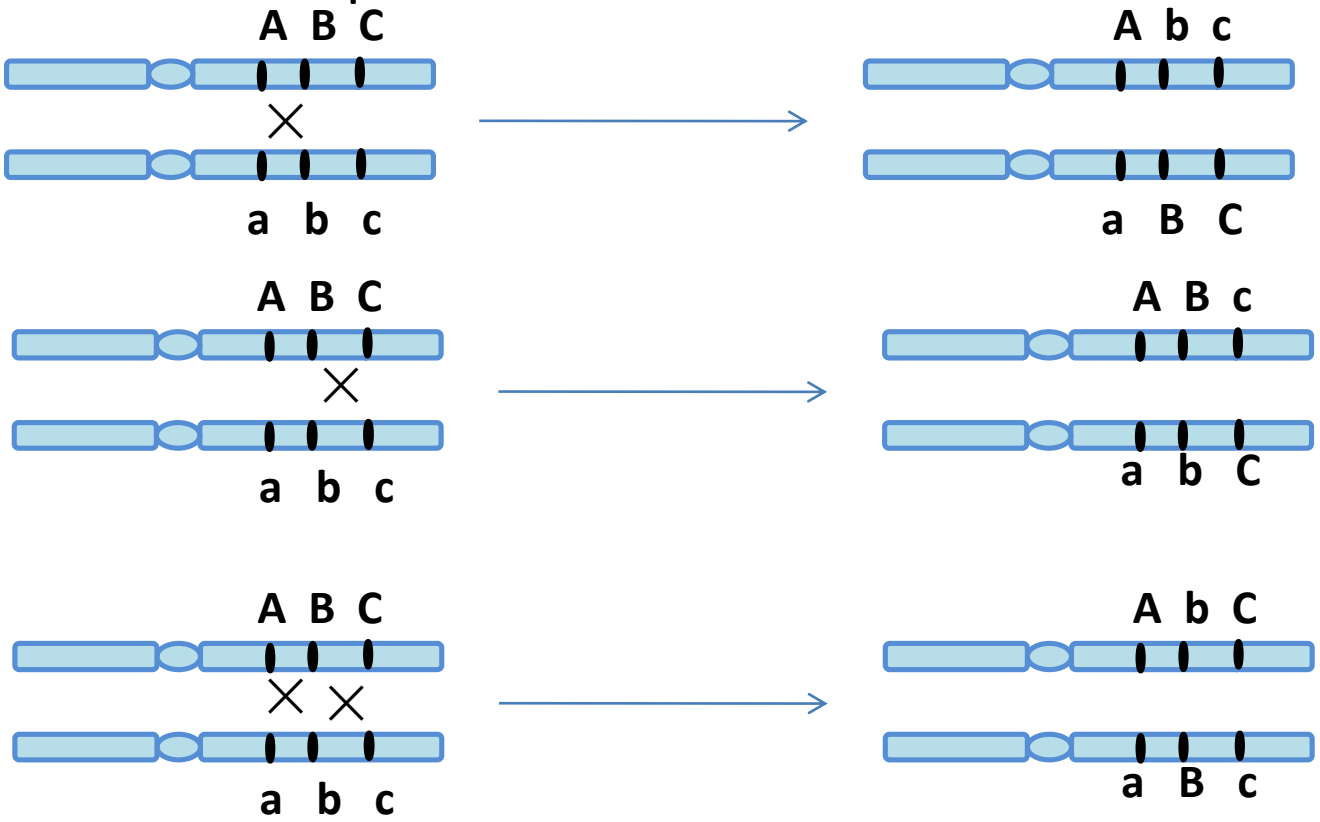
    Note: (Recombination rates i.e. map units are approximately additive)



Gene mapping from three point test cross

A cross involving three loci is referred to as three point cross

By adding third gene, there will be having several different types of crossover products that can be obtained



Possible types of recombination among 3 genes A, B and C

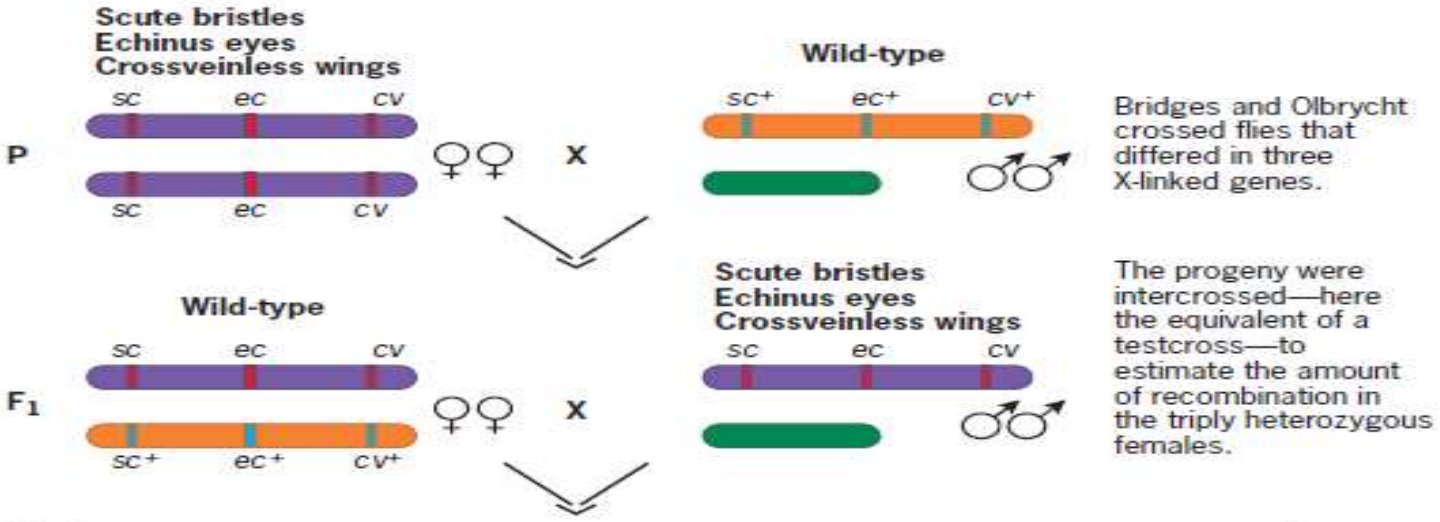
Example 1: Genes A,B and C are present on single chromosome (linked). A cross between individuals that are (**AABBCC**) and (**aabbcc**). Next, the F1 (**AaBbCc**) is testcrossed to an individual that is (**aabbcc**). The following data was obtained.

1. Determine which of the genotypes are parental genotype (The largest numbers)
2. Finding out double crossover (The least numbers)
3. Determine the order of genes (The middle gene will be moved from one sister chromatid to the other i.e. ABC→ ACB)
4. Determine the linkage distance between (A and C) and (C and B)
5. Distance between A and C = $\frac{81 + 85 + 5 + 8}{1000} \times 100 = 17.9\text{cM}$
6. Distance between C and B= $\frac{27 + 30 + 5 + 8}{1000} \times 100 = 7\text{cM}$
7. Gene order :

A 4cM **B** 7cM **C**

Genotype	Observed	Type of gamete
ABC	390	Parental
abc	374	Parental
AbC	27	Single crossover between genes C and B
aBc	30	Single crossover between C and B
ABc	5	Double crossover
abC	8	Double crossover
Abc	81	Single crossover between genes A and C
aBC	85	Single crossover between genes A and C
Total	1000	

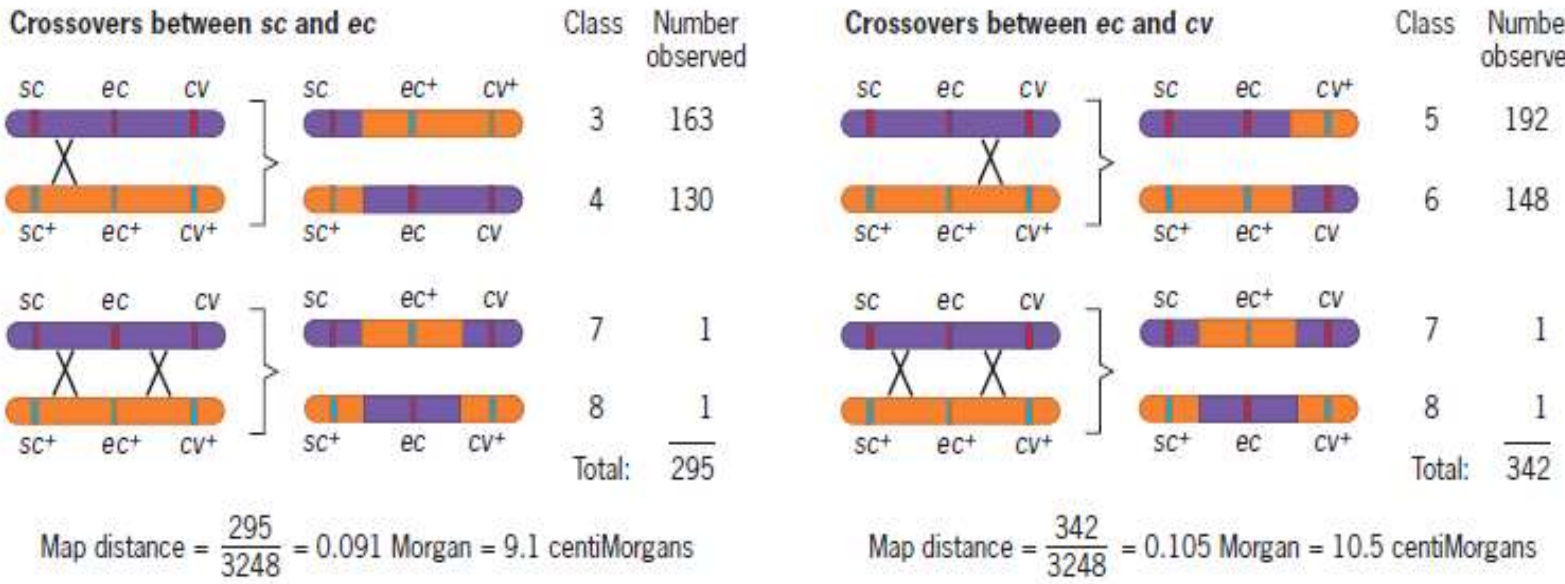
Example 2: Cross between wild type Drosophila male to female homozygous for three recessive X linked mutation- scute (sc) bristles, echinus (ec) eyes and crossveinless (cv) wings . They were intercrossed the F1 progeny to produce F2 flies, which they classified and counted.



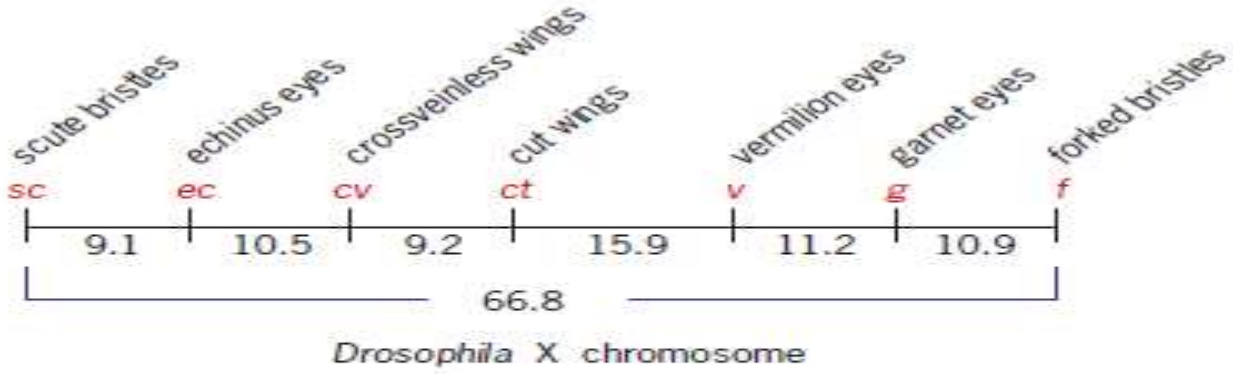
F_2

Class	*Phenotype*	*Genotype of maternally inherited X chromosome*			*Number observed*
1	Scute, echinus, crossveinless	*sc*	*ec*	*cv*	1158
2	Wild-type	*sc+*	*ec+*	*cv+*	1455
3	Scute	*sc*	*ec+*	*cv+*	163
4	Echinus, crossveinless	*sc+*	*ec*	*cv*	130
5	Scute, echinus	*sc*	*ec*	*cv+*	192
6	Crossveinless	*sc+*	*ec+*	*cv*	148
7	Scute, crossveinless	*sc*	*ec+*	*cv*	1
8	Echinus	*sc+*	*ec*	*cv+*	1
				Total:	3248

■ **FIGURE 7.12** Bridges and Olbrycht's three-point cross with the X-linked genes *sc* (*scute* bristles), *ec* (*echinus* eyes), and *cv* (*crossveinless* wings) in *Drosophila*. Data from Bridges, C. B., and Olbrycht, T. M., 1926. *Genetics* 11: 41.



■ **FIGURE 7.13** Calculation of genetic map distances from Bridges and Olbrycht's data. The distance between each pair of genes is obtained by estimating the average number of crossovers.



■ **FIGURE 7.14** Bridges and Olbrycht's map of seven X-linked genes in *Drosophila*. Distances are given in centiMorgans.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 142-145

Example 3 : An individual heterozygous for three genes, **AaBbCc** are testcrossed to **aabbcc** and 1000 progenies are classified by the gametic contribution of the heterozygous parent are as follows:

Draw a linkage map of the linked genes, showing the order and the distance in cM

ABc	abC	aBC	Abc	ABC	abc	aBc	AbC
305	310	42	43	140	145	6	9

1. Here Abc and abC are parental type
2. aBc and AbC are double recombinants types
3. Compare the parental and recombinants and determine gene order (gene order: BAC)
4. Rewrite the progeny in the table

Genotype	Number	Type
Bac	305	Parental type
baC	310	Parental type
BaC	42	SCO between A and B
bAc	43	SCO between A and B
BAC	140	SCO between A and C
Bac	145	SCO between A and C
Bac	6	DCO
bAC	9	DCO

Distance between 2 genes=

$$\frac{\text{Number of SCO} + \text{Number of DCO}}{\text{Total number of progeny}} \times 100\%$$

For A to B= $\frac{42 + 43 + 9 + 6}{1000} \times 100 = 10\text{cM}$

For A to C= $\frac{140 + 145 + 6 + 9}{1000} \times 100 = 30\text{cM}$

Map distance:

B 10 cM A 30 cM C

Interference and coincidence

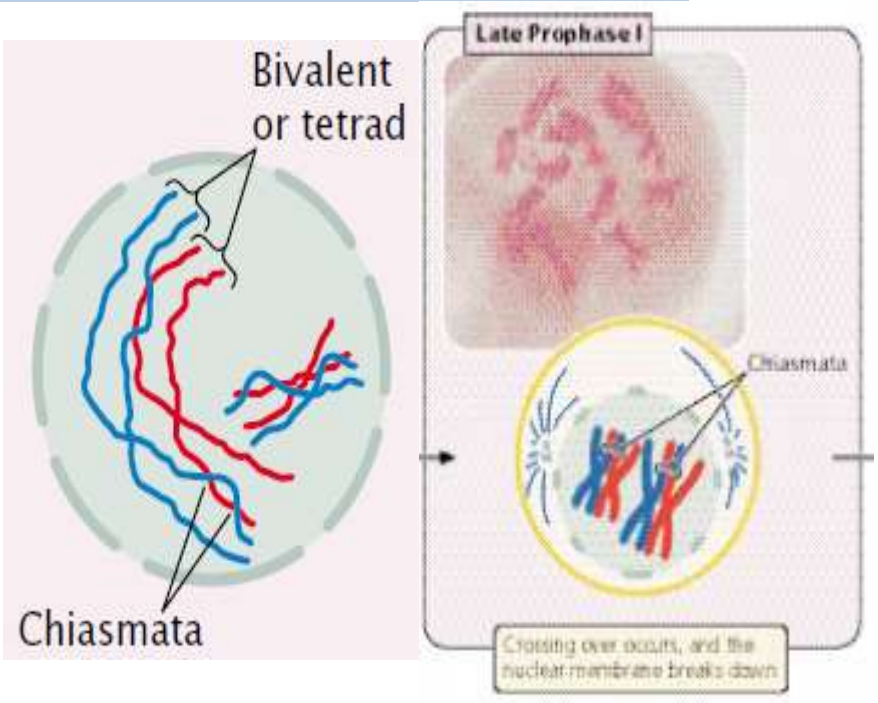
Crossing over →

- Not uniform across chromosome
- 1 Chiasma formation= reduces probability of another chiasma formation in immediately adjacent region

Lack of independence for formation of another chiasma= **Interference**

Net result of interference= observation of fewer double cross over types

- First observed by H J Muller and termed it as "Chiasma interference"
- The varying interference in different sections of chromosome is measured by- **Coefficient of coincidence**
- **Interference= 1- Coefficient of incidence**
  - COC=0 – Interference is complete, no double cross over
  - COC=Between 0 and 1- Partial interference
  - COC=1 No interference, expected double crossovers observed



Important formulas to be known

- Coefficient of coincidence(COC) = $\frac{\text{Number of observed double crossovers}}{\text{number of expected double crossovers}}$

- Expected Double crossover frequency=

  frequency of single cross over in 1st region × frequency of single crossover in 2nd region

- Number of expected double crossovers=

  Expected double crossovers frequency × total number of progeny

The Observed double crossover frequency= Lower than expected frequency

Because single crossover occurring at one region will reduce probability of 2nd single crossover at flanking region

Example: Suppose Drosophila females of genotype **AaBbCc** were crossed with males of genotype **aabbcc**. This led to 1000 progeny of the following type:

Genotype	Type
ABC	244 parental type
abc	236 parental type
ABc	81 SCO between B and C
abC	89 SCO between B and C
aBC	148 SCO between A and B
Abc	152 SCO between A and B
AbC	23 DCO
aBc	27DCO

Order of gene: ABC

1st region SCO Frequency between A and B=

$$\frac{152 + 148 + 27 + 23}{1000} = 0.35$$

2nd region frequency between B and C=

$$\frac{81 + 89 + 27 + 23}{1000} = 0.22$$

Expected DCO frequency= 0.35×0.22= 0.077

Number of expected DCO= 0.077×1000= 77

Observed DCO= 23+27= 50

COC= $\frac{50}{77} = 0.65$

Interference= 1- 0.65= 0.35